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Original Investigation

Effect of Chelation Therapy on Progressive Diabetic Nephropathy in Patients With Type 2 Diabetes and High-Normal Body Lead Burdens

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Background: A previous study in type 2 diabetic patients with high-normal body lead burdens showed that EDTA chelation therapy for 3 months slows progressive diabetic nephropathy during a 12-month follow-up. The effect of a longer course of therapy on kidney function decrease over a longer follow-up is not known.

Study Design: A 12-month run-in phase, then a randomized single-blind study with a 27-month intervention.

Setting & Participants: University medical center; 50 patients (serum creatinine, 1.5-3.9 mg/dL) with high-normal body lead burden (≥80-<600 μg) were randomly assigned to the treatment and control groups.

Intervention: The treatment group received weekly chelation therapy for 3 months to reduce their body lead burden to <60 μg and then as needed for 24 months to maintain this level. The control group received placebo for 3 months and then weekly for 5 weeks at 6-month intervals for 24 months.

Outcomes: The primary end point was change in estimated glomerular filtration rate (eGFR) over time. A secondary end point was a 2-fold increase in baseline serum creatinine level or the requirement for renal replacement therapy.

Measurements: Body lead burdens were assessed by EDTA mobilization tests and eGFR was calculated using the equation for Chinese patients with type 2 diabetes.

Results: Mean baseline eGFRs in the treatment and control groups were similar. After 3 months of chelation therapy, the change in eGFR in the treatment group (+1.0 ± 4.8 mL/min/1.73 m^2) differed significantly from that in the control group (−1.5 ± 4.8 mL/min/1.73 m^2; $P = 0.04$). In the subsequent 24-month intervention, the yearly rate of decrease in eGFR (5.6 ± 5.0 mL/min/1.73 m^2 per year) in the treatment group was slower than that (9.2 ± 3.6 mL/min/1.73 m^2 per year; $P = 0.04$) in the control group. 17 (68%) control-group patients and 9 (36%) treatment-group patients achieved the secondary end point.

Limitations: Small sample size, not double blind.

Conclusions: A 27-month course of EDTA chelation therapy retards the progression of diabetic nephropathy in type 2 diabetic patients with high-normal body lead burdens.

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INDEX WORDS: Progressive diabetic nephropathy; type 2 diabetes; repeated EDTA chelation therapy; body lead burden.

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Environmental exposure to lead, indicated by blood lead level, is thought to influence kidney function in healthy individuals.[1,2] Because blood lead level indicates only recent exposure to lead,[3,4] x-ray fluorescence methods and EDTA mobilization tests have been used to analyze body lead burden. Urinary lead excretion <600 μg/72 h after EDTA chelation therapy is considered to represent a normal body lead burden, whereas excretion of 80-600 μg/72 h is considered high-normal. Previous studies of nondiabetic patients with chronic kidney disease and normal body lead burdens[5-9] have suggested that environmental exposure to lead accelerates progressive decreases in kidney function and that repeated chelation therapy may retard disease progression in patients with high-normal body lead burdens.

During the past quarter century, the prevalence of type 2 diabetes has nearly doubled in the United States and has increased 3- to 5-fold worldwide.[10]

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Diabetes currently is the major cause of end-stage renal disease in both developed and emerging nations.[11,12] Previous studies have shown that bone lead content[13] or body lead burden,[14] assessed by EDTA mobilization tests, is associated with progressive loss of kidney function in diabetic patients. Moreover, a clinical trial indicated that EDTA chelation therapy retards progressive diabetic nephropathy in type 2 diabetic patients with high-normal body lead burdens.[14] However, the sample size of the trial was small (N = 30 patients), the duration of the trial was short (3 months of treatment with a 12-month follow-up), and estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease (MDRD) Study equation[15] rather than the Chinese equation,[16] which is a modified formula for calculating eGFR in Chinese patients. Hence, the role of EDTA chelation therapy in retarding diabetic nephropathy remains debatable. Because current treatments for diabetic nephropathy are of limited efficacy,[17] more effective treatment options are urgently required. Therefore, in the present study, we conducted a clinical trial in type 2 diabetic patients with high-normal body lead burden to determine whether a longer course of EDTA chelation therapy delays the progression of diabetic nephropathy during a 2-year period.

METHODS

Participants

The study protocol was approved by the Medical Ethics Committee of the Chang Gung Memorial Hospital (Taipei, Taiwan). Patients aged 30-83 years who had type 2 diabetes mellitus and received follow-up care at the Chang Gung Memorial Hospital for at least 1 year were eligible for inclusion in this study. Patients were recruited from the Nephrology Outpatient Department, and all patients with diabetic nephropathy were screened; a total of 86 individuals completed this 12-month run-in period.

Inclusion criteria for the randomized trial were as follows: serum creatinine concentration of 1.5-3.9 mg/dL, urinary protein excretion >0.5 g/d, diabetic retinopathy with laser therapy, pathologic diagnosis of diabetic nephropathy, absence of microhematuria, echograms showing normal-sized kidneys, history of diabetes for more than 5 years, no known history of exposure to lead or other heavy metals, and body lead burden of 80-600 μg, determined by the EDTA mobilization test in 72-hour urine samples.

Exclusion criteria were as follows: type 1 diabetes; decreased kidney function because of a potentially reversible cause, such as malignant hypertension, urinary tract infection, hypercalcemia, or drug-induced nephrotoxic effects; presence of other systemic diseases, such as connective-tissue diseases; use of drugs that might alter the course of the kidney disease, for example, nonsteroidal anti-inflammatory agents, steroids, immunosuppressive drugs, or Chinese herbal drugs; any drug allergy; and absence of a signed informed consent form.

The blood pressure of each patient was maintained at <140/90 mm Hg by administering diuretics and angiotensin-converting enzyme inhibitors or angiotensin receptor blockers, with or without calcium-blocking agents and/or vasodilators. Patients with systolic blood pressure <100 mm Hg were not administered angiotensin-converting enzyme inhibitors or angiotensin receptor blockers. Calcium carbonate was used to maintain patient phosphate levels. Because they had normal intact parathyroid hormone levels (<200 pg/mL), no patient was administered vitamin D_3 supplements or erythropoietin. All patients received dietary consultations and were recommended to follow a diabetic diet (35 kcal per kilogram of body weight per day) with normal protein intake (0.8-1.0 g of high-biological-value protein per kilogram of body weight per day). A nutritionist reviewed the dietary intake of each patient every 3-6 months. The 24-hour urea excretion levels were analyzed every 3 months to determine patient adherence to dietary recommendations.[18]

Measurement of Creatinine

Serum and urine creatinine levels in patients were determined by the alkaline picrate method of Jaffé[19] using an autoanalyzer (Hitachi 7600; Hitachi, www.hitachi.com). Bio-Rad Lyphochek control (Bio-Rad, www.bio-rad.com) was used as the creatinine reference standard to determine intrabatch accuracy and ensure interbatch standardization of the creatinine assay. The coefficient of variation for creatinine measurement was 2.6% at 1.4 mg/dL. External quality control was maintained by participation in the College of American Pathologists' Survey Program 3 times every year for 3 years. Both internal and external quality control procedures achieved consistently satisfactory results.

Measurement of Blood Lead Level and Body Lead Burden

Blood lead level and body lead burden were measured at the end of the run-in phase using a previously described method.[4-9] Body lead burden was measured using the EDTA mobilization test, modified by Behringer et al.[20] Urinary excretion was pooled over 72 hours after intravenous infusion of 1 g of calcium disodium EDTA (edetate calcium disodium [calcium disodium versenate]; Abbott Laboratories, www.abbott.com). Blood and urine lead levels were determined using electrothermal atomic-absorption spectrometry (SpectrAA-200Z; Agilent Technologies, www.agilent.com) with Zeeman background correction and L'vov platform. Both internal and external quality control procedures were applied throughout this study, and satisfactory results were consistently achieved. We used a certified and commercially manufactured product (Seronorm Trace Elements; Sero AS, www.sero.no) to monitor intrabatch accuracy and ensure interbatch standardization. The coefficient of variation for lead measurement was <5.3%. External quality control was maintained by participating in the governmental National Quality-Control Program.

Study Protocol

This study consisted of a 12-month run-in phase followed by a randomized single-blind study with a 27-month intervention and follow-up between August 2007 and October 2009.

The 12-Month Run-in Period

Serum creatinine, glycosylated hemoglobin (HbA_{1c}), daily urine protein excretion, daily protein intake, mean arterial pressure, and serum high- and low-density lipoprotein cholesterol levels were measured using an autoanalyzer system (model 736; Hitachi). These measurements were performed at the beginning and end and every 3 months during the 12-month run-in period. Patient blood pressure and body mass index also were measured at 3-month intervals. We collected laboratory data and 2 consecutive 24-hour urine samples from each patient and recorded the mean value of the 2 measurements. In the case of patients for whom urine collection data were incomplete (>1 missed urine collection) or who had inadequate urine flow (<1 mL/min), another urine

sample was collected. Kidney function was assessed by measuring creatinine clearance and eGFR (both expressed as milliliters per minute per 1.73 m^2). The formulas[16] for calculating eGFR in Chinese patients with type 2 diabetes ($R^2 = 0.95$) were eGFR (mL/min/1.73 m^2) = $313 \times age^{-0.494} \times [SCr]^{-1.059} \times [Alb]^{0.485}$ for men and eGFR (mL/min/1.73 m^2) = $783 \times age^{-0.489} \times [SCr]^{-0.877} \times [SUN]^{-0.150}$ for women, where age is in years, SCr is serum creatinine in milligrams per deciliter, Alb is serum albumin in grams per deciliter, and SUN is serum urea nitrogen level in milligrams per deciliter.

The 27-Month Intervention Period

The 12-month run-in period was followed by a 27-month, single-blind, randomized, placebo-controlled study. On the basis of previous studies,[4-9] we defined high-normal body lead burden as a minimum of 80 μg of lead (0.39 μmol) and a maximum of 600 μg (2.90 μmol). We randomly assigned 50 patients with high-normal body lead burden and serum creatinine level ≤3.9 mg/dL (≤353.6 μmol/L) to a control or treatment group. During the first 3 months, treatment-group patients received weekly 2-hour intravenous infusions of 1 vial (1 g) of calcium disodium EDTA mixed with 200 mL of normal saline solution until body lead burden was <60 μg (<0.29 μmol), whereas control patients received weekly 2-hour infusions of 1 vial (20 mL) of 50% glucose mixed with 200 mL of normal saline solution over 5 weeks.[9] Treatment-group patients were administered repeated lead chelation therapy if their serum creatinine levels exceeded prechelation baseline levels in the first 3 months and body lead burden was >60 μg or if body lead burden was >60 μg at their regular body lead burden reassessments every 6 months during the intervention period. Control patients were administered placebo weekly for 5 weeks every 6 months during the intervention period. In order to record possible changes in patient kidney function, laboratory measurements were conducted at 3-month intervals for an additional 24 months after the initial placebo or EDTA chelation therapy.

Outcome Measures

Temporal changes in kidney function (assessed by eGFR) of patients during the study period were considered to be the primary end point. An increase in serum creatinine level to 2-fold that of baseline level or need for renal replacement therapy was considered to be the secondary end point. As an analysis of the sensitivity of the primary end point measure, we included all patients in the analysis and assumed that those on renal replacement therapy had eGFR of 5 mL/min/1.73 m^2.

Sample Size Calculation

To detect mean differences between the treatment and control groups in rate of change in eGFR of 1.7 ± 0.6 (SD) mL/min/1.73 m^2 at 3-month intervals, which is in agreement with our previous study,[14] with a 2-sided 5% significance level and power >80%, the standard deviations of eGFR in the treatment and control groups ranging from 4.43-7.54 and 6.15-9.21 mL/min/1.73 m^2, respectively, and the expected variability (ie, standard deviation) within participants of 3.86 mL/min/1.73 m^2, a sample size of 18 patients per group was necessary, given an anticipated dropout rate of 20%. To recruit this number of patients, we anticipated needing a 12-month run-in period.

Statistical Analysis

Differences between groups were assessed using *t* tests for continuous variables and χ^2 tests for categorical variables. Mann-Whitney *U* test was used to assess variables without normal distribution. Differences in rates of progression of decreased kidney function between the 2 groups were analyzed using *t* tests and Mann-Whitney *U* tests. All *P* values were 2 tailed, and all results were presented as mean ± standard deviation values. Univariate analysis using Cox regression was performed to assess the effect of baseline variables on the secondary outcome. Variables with $P < 0.05$ in univariate analysis were entered into the Cox regression multivariable analysis for further evaluation. Data were presented as the hazard ratio (HR) with 95% confidence interval (CI). The cumulative percentage of all patients who reached the secondary end point was measured using the Kaplan-Meier method and log-rank test. $P < 0.05$ was considered to be statistically significant.

RESULTS

Run-in Period (Months 0-12)

A total of 86 patients completed the 12-month run-in phase, and body lead burden was measured at the end of this period. Sixty-five of these had high-normal body lead burdens (≥80-<600 μg), and after excluding those with serum creatinine level greater than the inclusion threshold and those who did not supply written informed consent, 50 patients (40 men and 10 women) who met the inclusion criteria participated in the clinical trial (Fig 1). In the 12-month run-in period prior to randomization, the yearly rate of decrease in eGFR in those later assigned to the treatment group was 10.1 ± 7.0 mL/min/1.73 m^2 per year, which was similar to that of the control group

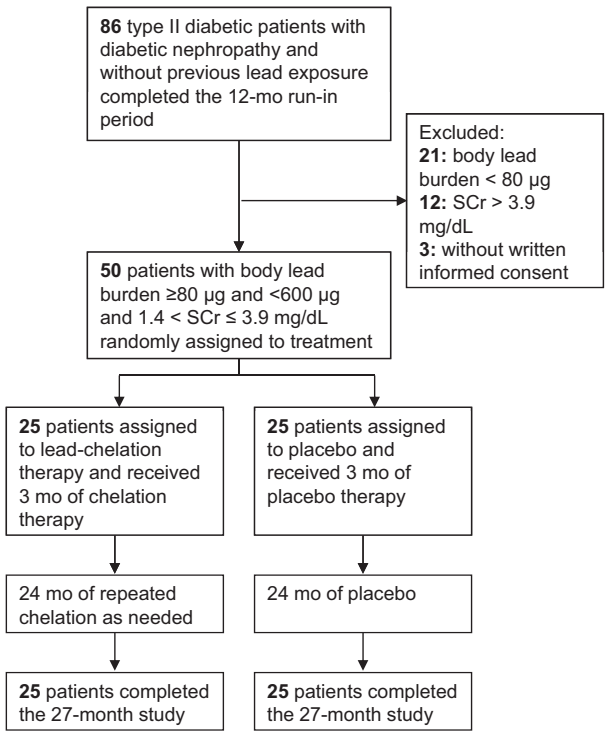

Figure 1. Flow chart of the enrollment and status of patients. Abbreviation: SCr, serum creatinine.

Table 1. Baseline Characteristics of Patients With High-Normal Body Lead Burden

Variable	Control Group (n = 25)	Treatment Group (n = 25)	*P*
Age (y)	56.1 ± 7.8 (43-66)	60.1 ± 12.8 (37-83)	0.2
Sex (male:female)	21:4	19:6	0.7[a]
Smoking	5 (20.0)	9 (36.0)	0.3[a]
Body mass index (kg/m^2)	26.1 ± 3.8 (21.3-32.2)	26.0 ± 2.7 (22.5-30.1)	0.9
Hyperlipidemia[b]	17 (68.0)	14 (56.0)	0.6[a]
Use of statin drugs	16 (64.0)	13 (52.0)	0.6[a]
Hypertension[c]	25 (100.0)	24 (96.0)	0.9[a]
Use of ACEi/ARB	25 (100.0)	25 (100.0)	0.9[a]
Use of nondihydropyridine CCB	7 (28.0)	11 (44.0)	0.4[a]
Use of dihydropyridine CCB	8 (32.0)	11 (44.0)	0.6[a]
Previous cardiovascular disease[d]	5 (20.0)	6 (24.0)	0.9[a]
Retinopathy with laser therapy	25 (100.0)	24 (96.0)	0.9[a]
Use of insulin at entry	17 (68.0)	17 (68.0)	0.9[a]
Hemoglobin A_{1c} (%)	8.3 ± 2.4 (5.4-13.0)	8.2 ± 1.6 (5.8-12.8)	0.7
Mean arterial pressure (mm Hg)	99.8 ± 10.4 (77.7-116.3)	98.7 ± 10.7 (80.0-116.0)	0.7
HDL cholesterol (mg/dL)	38.8 ± 3.8 (34-50)	38.2± 5.8 (26-54)	0.7
LDL cholesterol (mg/dL)	148.0± 47.9 (83-224)	131.7 ± 40.7 (54-217)	0.5
Serum creatinine (mg/dL)	2.9 ± 0.6 (1.8-3.7)	2.8 ± 0.7 (1.9-3.9)	0.6
CCr (mL/min/1.73 m^2)	27.5 ± 7.6 (18.0-42.0)	30.3 ± 13.4 (13.9-60.8)	0.4
eGFR[e] (mL/min/1.73 m^2)	29.5 ± 6.2 (21.9-43.5)	27.6± 4.7 (17.0-32.8)	0.2
Blood lead (μg/dL)	6.3± 2.4 (1.8-12.4)	7.1 ± 4.1 (1.8-17.0)	0.4
Body lead burden (μg)	151.3 ± 93.6 (81.0-373.0)	142.1 ± 39.2 (95.0-217.4)	0.6
Daily protein excretion (g)	4.0 ± 2.2 (0.5-7.7)	3.8 ± 2.7 (0.5-12.4)	0.7
Daily protein intake (g/kg)	0.91 ± 0.21 (0.46-1.17)	0.87 ± 0.20 (0.51-1.16)	0.4

Note: A high-normal body lead burden was defined as lead level of at least 80 μg (0.39 μmol), but <600 μg (<2.9 μmol). Values for continuous variables given as mean ± standard deviation (range); values for categorical variables given as number (percentage). Conversion factors for units: serum creatinine in mg/dL to μmol/L, ×88.4; cholesterol in mg/dL to mmol/L, ×0.02586; lead in μg/dL to μmol/L, ×0.04286.

Abbreviations: ACEi, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor antagonists; CCB, calcium channel blocker; CCr, creatinine clearance rate; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

[a]*P* values were calculated by χ^2 test, except in the comparisons of age, body mass index, serum creatinine, CCr, GFR, blood lead level, and body lead burden, which were calculated by *t* test.

[b]Hyperlipidemia was defined as serum cholesterol level >240 mg/dL (>6.2 mmol/L) after diet control.

[c]Hypertension was defined by the presence of at least 2 blood pressure measurements ≥140/90 mm Hg in a patient who was receiving antihypertensive drugs.

[d]Previous cardiovascular disease included ischemic heart disease, congestive heart failure, peripheral vascular disease, and cerebrovascular disease.

[e]eGFR calculated by the formula for Chinese with type 2 diabetes.

(11.2 ± 6.7 mL/min/1.73 m^2 per year; $P = 0.3$, Mann-Whitney *U* test).

Intervention Period (Months 0-27)

Initial Chelation Therapy (Months 0-3)

Both groups had similar baseline characteristics (Table 1). After the 3 months of lead chelation therapy, the body lead burden of treatment-group patients decreased to 49.9 ± 18.7 (range, 9.2-68.0) μg, and blood lead levels decreased to 3.4 ± 1.9 (range, 1.3-9.1) μg/dL. The average therapeutic dose of calcium disodium EDTA was 5.6 ± 2.6 (range, 3-12) g. After the initial chelation therapy, an improvement in kidney function of treatment-group patients was observed (Table 2; change in eGFR, +1.0 ± 4.8 mL/min/1.73 m^2 in the treatment group vs −1.5 ± 4.8 mL/min/1.73 m^2 in the control group; $P = 0.04$).

Repeated Chelation Therapy (Months 4-27)

The 2 groups did not differ in terms of body mass index; mean arterial pressure; levels of serum HbA_{1c}, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol; daily urinary protein excretion; or daily protein intake (Table 3) throughout the intervention period. During this period, 5 patients in the treatment group required

Table 2. Kidney Function During the Run-in and Clinical Trial Periods

	Control Group (n = 25)	Treatment Group (n = 25)	Mean Difference (95% CI)	*P*
Run-in Period (Month −12 to Month 0)				
eGFR				
Month −12	40.8 ± 9.4	37.7 ± 5.4	3.07 (−1.3 to 7.4)	0.2
Month −9	37.4 ± 9.5	34.3 ± 6.7	3.10 (−1.6 to 7.8)	0.2
Month −6	33.4 ± 9.4	33.1 ± 7.0	0.37 (−4.3 to 5.1)	0.9
Month −3	30.7 ± 5.5	30.6 ± 4.8	0.07 (−2.9 to 3.0)	0.9
Month 0	29.5 ± 5.9	27.6 ± 4.7	1.90 (−1.1 to 4.9)	0.2
Rate of eGFR decrease, months −12 to 0 (mL/min/1.73 m^2 per y)	11.2 ± 6.7	10.1 ± 7.0	0.86 (−5.1 to 2.7)	0.3[a]
Lead Chelation and Follow-up Period (Month 0 to Month 27)				
ΔeGFR, months 0-3	−1.5 ± 4.8	+1.0 ± 4.8	−2.55 (−5.2 to 0.2)	0.04[a]
eGFR				
Month 3	28.0 ± 7.3	28.6 ± 6.7	−0.60 (−4.6 to 3.4)	0.8
Month 6	23.7 ± 5.5	26.8 ± 7.0	−3.12 (−6.7 to 0.5)	0.09
Month 9	21.9 ± 5.5	24.0 ± 6.5	−2.14 (−5.6 to 1.3)	0.2
Month 12	19.1 ± 5.7	24.6 ± 8.9	−5.49 (−9.7 to −1.2)	0.01
Month 15	17.1 ± 6.3	22.8 ± 9.4	−5.69 (−10.3−1.1)	0.02
Month 18	15.4 ± 6.3	21.6 ± 9.4	−6.13 (−10.7 to −1.6)	0.01
Month 21	13.0 ± 6.6[b]	20.0 ± 9.9	−7.00 (−11.8 to −2.2)	0.005
Month 24	11.7 ± 7.2[c]	18.0 ± 10.8[d]	−6.37 (−11.6 to −1.2)	0.02
Month 27	9.7 ± 6.7[e]	17.4 ± 11.4[f]	−7.64 (−12.0 to −1.8)	0.006
Rate of eGFR decrease, months 3-27 (mL/min/1.73 m^2 per y)	9.2 ± 3.6	5.6 ± 5.0	3.52 (1.0 to 6.0)	0.04[a]
ΔeGFR, months 0-27	−19.8 ± 7.4	−10.3 ± 11.6	9.54 (4.0 to 15.1)	0.009[a]

Note: Values given are mean eGFR±standard deviation (eGFR calculated using equation for Chinese with type 2 diabetes and given in mL/min/1.73 m^2) or mean difference (control group value minus treatment group value, in mL/min/1.73 m^2). Data were analyzed by *t* test; significant differences are those with $P < 0.05$. Months 0-3 constitute the initial chelation period; months 3-27 constitute the repeated lead chelation and follow-up period.

Abbreviations: ΔeGFR, change in estimated glomerular filtration rate; CI, confidence interval.

[a]Measured by Mann-Whitney *U* tests.
[b]n = 20.
[c]n = 19.
[d]n = 23.
[e]n = 14.
[f]n = 21.

only one course of repeated chelation therapy, whereas 8 required 2 courses, 10 required 3 courses, and 2 required 4 courses. The mean dose of calcium disodium EDTA administered to patients during repeated chelation therapy was 5.2 ± 2.7 (range, 3-9) g.

At the end of this study, blood lead level (3.8 ± 1.2 μg/dL) and body lead burden (46.0 ± 23.1 μg) of treatment-group patients were lower than blood lead level (6.8 ± 3.1 μg/dL; $P = 0.03$) and body lead burden (151.3 ± 93.6 μg; $P < 0.001$) of control patients. At this stage, the eGFR of treatment-group patients (17.4 ± 11.4 mL/min/1.73 m^2) was higher than that of the control group (9.7 ± 6.7 mL/min/1.73 m^2; $P = 0.006$; Table 2). The yearly rate of decrease in eGFR of the treatment group during this period was 5.6 ± 5.0 mL/min/1.73 m^2, which was less than that of the control group (9.2 ± 3.6 mL/min/1.73 m^2; $P = 0.04$; Table 2; Fig 2). No side effects of repeated lead chelation therapy were observed during the 27-month study period. Twenty-six patients, including 17 (68%) control-group patients and 9 (36%) treatment-group patients, reached the secondary end point during the study period ($P = 0.02$, log-rank test; Fig 3). Of these 26 patients who showed a 2-fold increase in baseline serum levels, 11 (44%) control-group patients and 4 (16%) treatment-group patients received renal replacement therapy.

As a sensitivity test, if an eGFR of 5 mL/min/1.73 m^2 was assumed for kidney function in patients with renal replacement therapy, similar results were obtained. At the end of the clinical trial, the eGFR of treatment-group patients (16.0 ± 11.4 mL/min/1.73 m^2) was higher than that of the control group (8.3 ± 6.0 mL/min/1.73 m^2; $P < 0.001$). The yearly rate of decrease in eGFR in the treatment group during this period was 6.4 ± 5.0 mL/min/1.73 m^2, which was less than that of the control group (9.8 ± 3.2 mL/min/1.73 m^2, $P = 0.02$).

Multivariable Cox analysis showed that EDTA treatment reduced the risk (HR, 0.39; 95% CI, 0.16-0.93; $P = 0.03$) of the secondary outcome during the 27-month study period (Table 4).

Table 3. Mean Values of Variables Related to Kidney Function During the Clinical Trial Period

	Control Group	Treatment Group	*P*
Month 3			
Body mass index (kg/m^2)	25.7 ± 3.5	26.3 ± 3.4	0.6
Mean arterial pressure (mm Hg)	97.8 ± 10.3	99.3 ± 10.3	0.6
HbA_{1c} (%)	8.6 ± 2.1	8.7 ± 2.1	0.9
HDL cholesterol (mg/dL)	39.2 ± 4.7	40.2 ± 5.7	0.5
LDL cholesterol (mg/dL)	118.4 ± 27.0	125.1 ± 40.0	0.5
Daily urine protein (g)	3.3 ± 2.1	3.6 ± 2.1	0.7
Daily protein intake (g/kg)	0.93 ± 0.14	0.95 ± 0.09	0.6
Month 9			
Body mass index (kg/m^2)	26.1 ± 4.2	26.1 ± 2.9	0.9
Mean arterial pressure (mm Hg)	98.1 ± 4.7	95.6 ± 10.6	0.3
HbA_{1c} (%)	8.3 ± 2.4	9.1 ± 2.1	0.3
HDL cholesterol (mg/dL)	38.0 ± 5.1	40.0 ± 7.9	0.3
LDL cholesterol (mg/dL)	124.0 ± 26.4	119.3 ± 29.0	0.6
Daily urine protein (g)	4.0 ± 2.3	3.6 ± 2.2	0.6
Daily protein intake (g/kg)	0.87 ± 0.23	0.83 ± 0.17	0.6
Month 15			
Body mass index (kg/m^2)	26.3 ± 4.1	25.6 ± 3.4	0.5
Mean arterial pressure (mm Hg)	95.1 ± 7.3	93.8 ± 8.7	0.6
HbA_{1c} (%)	7.7 ± 2.0	8.3 ± 2.7	0.2
HDL cholesterol (mg/dL)	38.0 ± 3.7	37.9 ± 5.8	0.9
LDL cholesterol (mg/dL)	132.9 ± 48.0	126.9 ± 31.1	0.6
Daily urine protein (g)	4.2 ± 2.8	3.8 ± 2.8	0.6
Daily protein intake (g/kg)	0.80 ± 0.16	0.84 ± 016	0.6
Month 21[a]			
Body mass index (kg/m^2)	26.3 ± 4.3	25.7 ± 3.2	0.5
Mean arterial pressure (mm Hg)	93.9 ± 6.7	91.6 ± 11.7	0.4
HbA_{1c} (%)	8.2 ± 2.4	9.0 ± 2.1	0.3
HDL cholesterol (mg/dL)	37.9 ± 3.6	37.7 ± 5.7	0.9
LDL cholesterol (mg/dL)	131.6 ± 36.0	124.2 ± 32.2	0.6
Daily urine protein (g)	4.0 ± 2.7	3.4 ± 2.2	0.4
Daily protein intake (g/kg)	0.88 ± 0.26	0.81 ± 0.13	0.2
Month 27[b]			
Body mass index (kg/m^2)	26.4 ± 3.9	25.8 ± 3.3	0.5
Mean arterial pressure (mm Hg)	93.2 ± 7.2	90.6 ± 8.7	0.2
HbA_{1c} (%)	8.7 ± 2.3	8.1 ± 1.9	0.3
HDL cholesterol (mg/dL)	40.5 ± 4.9	39.2 ± 4.2	0.5
LDL cholesterol (mg/dL)	126.6 ± 32.8	120.8 ± 20.1	0.4
Daily urine protein (g)	4.1 ± 2.4	3.6 ± 2.3	0.5
Daily protein intake (g/kg)	0.81 ± 0.15	0.85 ± 0.18	0.4

Note: Conversion factor for cholesterol in mg/dL to mmol/L, ×0.02586.
Abbreviations: HbA_{1c}, hemoglobin A_{1c}; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
[a]n = 20 for the control group.
[b]n = 14 for the control group; n = 21 for the study group.

DISCUSSION

Similar to previous studies,[4-9,14,21,22] the data we present here show that EDTA therapy may delay the progression of diabetic nephropathy. In the present study, we enrolled a new cohort that was not included in our previous studies.[14] There also were several differences from our previous studies that allowed us to make a number of new observations and draw more definitive conclusions about the effects of EDTA treatment in retarding the progression of diabetic nephropathy. First, the present study differed from our previous studies with respect to the definition of the secondary end point (serum creatinine level 2-fold vs 1.5-fold that of baseline), follow-up period (27 vs 15 months), sample size (50 vs 30 participants), and treatment method (repeated EDTA treatment vs no treatment during the follow-up period). Second, multivariable Cox analysis shows that EDTA chelation therapy results in a 61% decrease in risk of achieving the secondary outcome. Third, although our sample size was small (N = 50), we found that statistical power was >0.80. However, because we had included

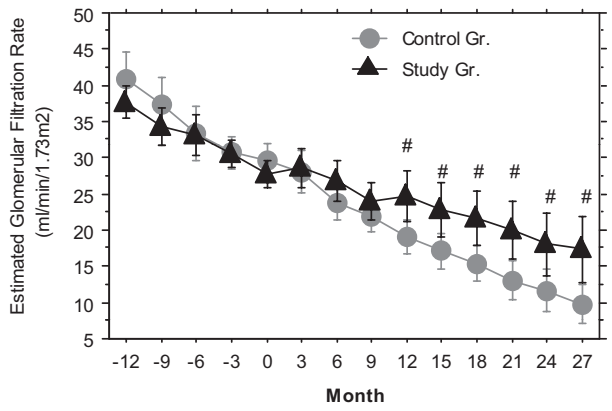

Figure 2. Estimated mean glomerular filtration rate over time in the chelation group (Gr.; n = 25) and control group (n = 25) during the study period. Patients in the study group received chelation therapy from month 0 to month 27. *$P < 0.05$ by *t* tests between the 2 groups on months 12 and 27 (±2 SE). (Month 12, $P = 0.01$; month 15, $P = 0.02$; month 18, $P = 0.01$; month 21, $P = 0.005$; month 24, $P = 0.02$; and month 27, $P = 0.006$.)

only patients with a high-normal body lead burden in this study and hence had only a small sample size, we could not show that body lead burden predicts changes in kidney function.

The mechanism by which EDTA chelation therapy retards progressive diabetic nephropathy is unclear. However, plasma nitric oxide (NO) levels are increased after EDTA administration and there is greater and more diffuse endothelial NO synthase expression in kidneys of rats treated with EDTA than in control animals.[23] In addition, EDTA administration prevents tumor necrosis factor α–induced renal vascular leakage and protects rat kidneys from ischemic damage in vivo, potentially by stimulation of NO production.[23] EDTA chelation therapy also can remove contaminating metals and decrease free radical production.[24,25] This therapy exerts long-term antioxidant effects because plasma peroxide levels in patients have been observed to decrease persistently.[25] Moreover, repeated EDTA chelation therapy protects cellular lipids against oxidative damage.[25,26] Lead chelation reduces levels of reactive oxygen species, enhances the availability of NO to vascular smooth muscle cells, and decreases blood pressure in rats with lead-induced hypertension.[27] Hence, improvements in kidney function in our patients after EDTA treatment may be due to the antioxidative effects of the therapy in addition to the removal of lead.

The EDTA chelating agent, administered at smaller doses and over longer intervals, has been used safely to treat patients with chronic kidney disease.[5-9,14,21,28-30] The mean dose of EDTA used in the present study was only 5.6 g in the initial 3 months and 5.2 g in the 24-month follow-up. This dosage is much lower than that used for treating patients with acute lead poisoning, who are administered 40-60 g of EDTA within 2-3 weeks. Some researchers argue that lead redistribution resulting from EDTA chelation therapy may increase concentrations of lead in the brain, thereby causing brain damage. However, animal studies[24,31] have not shown an increase in brain lead concentrations after EDTA chelation therapy. Stangle et al[32] showed that 2,3-dimercaptosuccinic acid chelation impairs cognitive ability in 30-day-old rats without lead exposure. However, the dose of chelating agent in that study was as large as that used to treat acute poisoning and was given over a short period (3 weeks). Therefore, it is not surprising that the chelating agent may have induced cognitive impairment in these very young animals.[32]

One of the limitations of the present study was the use of eGFR to assess kidney function. However, studies by Leung et al[16] of eGFR in Chinese patients with type 2 diabetes showed a strong correlation between eGFR and GFR measured by an isotopic method ($r^2 = 0.95$). Another limitation was that body lead burden was assessed using the EDTA mobilization test. Lead that can be chelated predominantly reflects lead concentrations in blood, soft tissues, and a fraction of the trabecular bone, but not cortical bone.[33] Because kidneys are included in soft tissues, EDTA mobilization may reflect lead content of the kidney,[24] which may influence progressive chronic kidney disease. Measurements of bone lead with x-ray fluorescence methods may be more useful to assess body lead burden than the EDTA mobilization test. However, there are several important limitations to measurements of bone lead concentrations by x-ray fluorescence methods,[33] such as lack of precision, nonhomogenous lead distribution in cortical bone, and a low turnover rate with low biological activity of lead in cortical bone. Glucose may interfere with the Jaffé method to measure creatinine.[34] Although HbA_{1c}

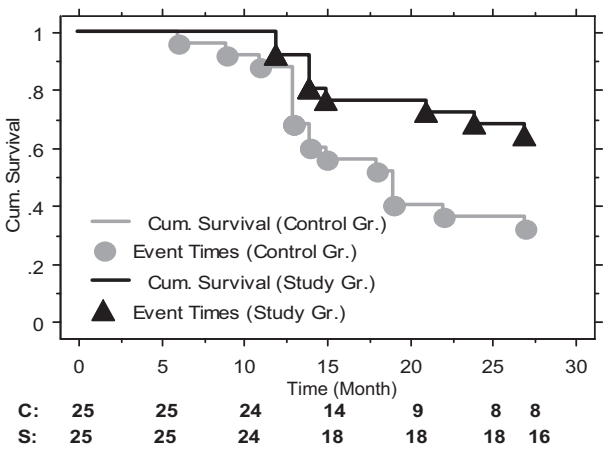

Figure 3. Kaplan-Meier analysis of the cumulative (Cum.) percentage of patients with the secondary outcome. (Log-rank test, $P = 0.02$). Abbreviations: C, control group; Gr., group; S, study group.

Table 4. Regression Analysis of the Overall Risk of the Secondary Outcome of Progressive Diabetic Nephropathy

Variable	Univariate Cox Analysis	*P*	Multivariable Cox Analysis	*P*
Baseline				
Age (per 1-y increase)	0.96 (0.93-1.00)	0.04	1.01 (0.96-1.07)	0.5
Sex (male)	1.26 (0.48-3.36)	0.6		
Smoking (yes)	1.80 (0.68-4.79)	0.2		
Body mass index (per 1-kg/m^2 increase)	1.06 (0.94-1.20)	0.4		
Hyperlipidemia (yes)	1.47 (0.64-3.39)	0.4		
Hypertension (yes)	1.47 (0.64-3.39)	0.7		
Previous cardiovascular diseases (yes)	1.40 (0.48-4.08)	0.5		
Use of insulin injection (yes)	1.20 (0.53-2.69)	0.7		
HbA_{1c} (per 1% increase)	1.11 (0.93-1.32)	0.3		
HDL cholesterol (per 1-mg/dL greater)	1.05 (1.00-1.11)	0.08		
LDL cholesterol (per 1-mg/dL greater)	1.00 (1.00-1.01)	0.5		
Serum creatinine (per 1-mg/dL greater)	1.83 (1.08-3.10)	0.02	1.01 (0.96-1.07)	0.2
Body lead burden (per 1-μg greater)	1.00 (1.00-1.01)	0.6		
Daily urine protein excretion (per 1-g greater)	1.15 (1.02-1.29)	0.03	1.28 (1.06-1.54)	0.08
Daily protein intake (per 1-g/kg/d greater)	0.66 (0.09-4.88)	0.3		
Lead chelation therapy (yes)	0.40 (0.18-0.89)	0.03	0.39 (0.16-0.93)	0.03

Note: Except where indicated, values are given as hazard ratio (95% confidence interval).
Abbreviations: HbA_{1c}, hemoglobin A_{1c}; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

levels were similar between the control and treatment groups in the present study, the use of methods[35] to improve specificity and minimize susceptibility to interfering substances is required in future studies. Another limitation of our study was that it had an incomplete single-blind placebo design and there may have been unrecognized differences in care, such as antihypertensive drug treatment and dietary counseling, between the 2 assigned treatment groups. However, no differences in blood pressure, HbA_{1c} levels, or blood lipid levels were noted between the 2 treatment groups during the course of the study. Because ~70% of patients with diabetic nephropathy have been found to have a high-normal body lead burden (80-600 μg) in a previous (61/87; 70.1%) and the present study (65/86; 75.6%), the results we present here might be generalizable to the larger diabetic nephropathy population.

In conclusion, our study results indicate that when other treatable factors are controlled, EDTA chelation treatment may delay the rapid progression of diabetic nephropathy in type 2 diabetic patients with high-normal body lead burdens. Our results may be important because diabetic nephropathy is a major global cause of end-stage renal disease.

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REFERENCES

1. Staessen JA, Lauwerys RR, Buchet J-P, et al. Impairment of renal function with increasing blood lead concentrations in the general population. *N Engl J Med*. 1992;327(3):151-156.

2. Kim R, Rotnitsky A, Sparrow D, Weiss S, Wager C, Hu H. A longitudinal study of low level lead exposure and impairment of renal function: the Normative Aging Study. *JAMA*. 1996;275(15):1177-1181.

3. Pollock CA, Ibels LS. Lead nephropathy—a preventable cause of renal failure. *Int J Artif Organs*. 1988;11(2):75-78.

4. Lin JL, Yeh KH, Tseng HC, Chen WY, Lai HH, Lin YC. Urinary *N*-acetyl-glucosaminidase excretion and environmental lead exposure. *Am J Nephrol*. 1993;13(6):442-447.

5. Lin JL, Ho HH, Yu CC. Chelation therapy for patients with elevated body lead burden and progressive renal insufficiency: a randomized, controlled trial. *Ann Intern Med*. 1999;130(1):7-13.

6. Lin JL, Tan DT, Hsu KH, Yu CC. Environmental lead exposure and progressive renal insufficiency. *Arch Intern Med*. 2001;161(2):264-271.

7. Yu CC, Lin JL, Lin-Tan DT. Environmental exposure to lead and progression of chronic renal disease: a four-year prospective longitudinal study. *J Am Soc Nephrol*. 2004;15(4):1016-1022.

8. Lin JL, Tan DT, Ho HH, Yu CC. Environmental lead exposure and urate excretion in the general population. *Am J Med*. 2002;113(7):563-568.

9. Lin JL, Lin-Tan DT, Hsu KH, Yu CC. Environmental lead exposure and progression of chronic renal diseases in patients without diabetes. *N Engl J Med*. 2003;348(4):277-286.

10. Yoon KH, Lee JH, Kim JW, et al. Epidemic obesity and type 2 diabetes in Asia. *Lancet*. 2006;368(9548):1681-1688.

11. US Renal Data System. International comparisons. In: USRDS. *USRDS 2007 Annual Data Report: Atlas of Chronic Kidney Disease and End-Stage Renal Disease in the United States.* Bethesda, MD: National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases; 2007:239-254.

12. US Renal Data System. *USRDS 1999 Annual Data Report.* Bethesda, MD: National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases; 1999:25-38.

13. Tsaih SW, Korrick S, Schwartz J, et al. Lead, diabetes, hypertension, and renal function: the Normative Aging Study. *Environ Health Perspect*. 2004;112(11):1178-1182.

14. Lin JL, Lin-Tan DT, Yu CC, Huang YY, Li KL. Environmental exposure to lead and progressive diabetic nephropathy in patients with type II diabetes. *Kidney Int*. 2006;69(11):2049-2056.

15. Levey AS, Bosch JP, Lewis JB, et al. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. *Ann Intern Med*. 1999;130(6):461-470.

16. Leung TK, Luk AO, So WY, Lo MK, Chan JC. Development and validation of equations estimating glomerular filtration rates in Chinese patients with type 2 diabetes. *Kidney Int*. 2010; 77(8):729-735.

17. Burney B, Kalaitzidis R, Bakris G. Novel therapies of diabetic nephropathy. *Curr Opin Nephrol Hypertens*. 2009;18(2): 107-111.

18. Isaksson B. Urinary nitrogen output as a validity test in dietary surveys. *Am J Clin Nutr*. 1980;33(1):4-5.

19. Gerard SK, Khayam-Bashi H. Characterization of creatinine error in ketotic patients: a prospective comparison of alkaline picrate methods with an enzymatic method. *Am J Clin Pathol*. 1985;84(5):659-664.

20. Behringer D, Craswell P, Mohl C, Stoeppler M, Ritz E. Urinary lead excretion in uremic patients. *Nephron*. 1986;42(4):323-329.

21. Wedeen RP, Mallick DK, Batuman V. Detection and treatment of occupational lead nephropathy. *Arch Intern Med*. 1979; 139(1):53-57.

22. Khalil-Manesh F, Gonick HC, Cohen A, et al. Experimental model of lead nephropathy. II. Effect of removal from lead exposure and chelation treatment with dimercaptosuccinic acid (DMSA). *Environ Res*. 1992;58(1):35-54.

23. Foglieni C, Fulgenzi A, Ticozzi P, et al. Protective effect of EDTA preadministration on renal ischemia. *BMC Nephrol*. 2006;7:5.

24. Sanchez-Fructuoso AI, Cano M, Arroyo M, Fernandez C, Prats D, Barrientos A. Lead mobilization during calcium disodium ethylenediaminetetraacetate chelation therapy in treatment of chronic lead poisoning. *Am J Kidney Dis*. 2002;40(1):51-58.

25. Hininger I, Waters R, Osman M, et al. Acute prooxidant effects of vitamin C in EDTA chelation therapy and long-term antioxidant benefits of therapy. *Free Radic Biol Med*. 2005;38(12):1565-1570.

26. Jacobsen C, Hartvigsen K, Thomsen MK, et al. Lipid oxidation in fish oil enriched mayonnaise: calcium disodium ethylenediaminetetraacetate, but not gallic acid, strongly inhibited oxidative deterioration. *J Agric Food Chem*. 2001;49(2):1009-1019.

27. Ding Y, Vaziri ND, Gonick HC. Lead-induced hypertension. II. Response to sequential infusions of L-arginine, superoxide dismutase, and nitroprusside. *Environ Res*. 1998;76(2):107-113.

28. Wedeen RP, Batuman V, Landy E. Safety of EDTA lead mobilization test. *Environ Res*. 1983;30(1):58-62.

29. Lin-Tan DT, Lin JL, Yen TH, Chen KH, Huang YL. Long-term outcome of repeated lead chelation therapy in progressive non-diabetic chronic kidney diseases. *Nephrol Dial Transplant*. 2007;22(10):2924-2931.

30. Lin JL, Lin-Tan DT, Li YJ, Chen KH, Huang YL. Low-level environmental exposure to lead and progressive chronic kidney diseases. *Am J Med*. 2006;119(8):707.e1-e9.

31. Sánchez-Fructuoso AI, Blanco J, Cano M, et al. Experimental lead nephropathy: treatment with calcium disodium ethylenediaminetetraacetate. *Am J Kidney Dis*. 2002;40(1):59-67.

32. Stangle DE, Smith DR, Beaudin SA, Strawderman MS, Levitsky DA, Strupp BJ. Succimer chelation improves learning, attention, and arousal regulation in lead-exposed rats but produces lasting cognitive impairment in the absence of lead exposure. *Environ Health Perspect*. 2007;115(2):201-209.

33. Barbosa F Jr, Tanus-Santos JE, Gerlach RF, Parsons PJ. A critical review of biomarkers used for monitoring human exposure to lead: advantages, limitations, and future needs. *Environ Health Perspect*. 2005;113(12):1669-1674.

34. Da Rin G, Amici G, Virga G, Bardin C, Calzavara P, Bocci C. Correction of glucose concentration interference on Jaffé kinetic creatinine assay in peritoneal dialysis. *Am J Nephrol*. 1995; 15(6):480-487.

35. Myers GL, Miller WG, Coresh J, et al; National Kidney Disease Education Program Laboratory Working Group. Recommendations for improving serum creatinine measurement: a report from the Laboratory Working Group of the National Kidney Disease Education Program. *Clin Chem*. 2006;52(1):5-18.